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Purification and characterization of membrane-bound phosphatidylinositol kinase from rat brain.

Yamakawa A, Takenawa T.

Department of Pharmacology, Tokyo Metropolitan Institute of Gerontology, Japan.

A membrane-bound phosphatidylinositol (PI) kinase was purified from rat brain. The enzyme was solubilized with Triton X-100 from salt-washed membrane and purified 11,183-fold, with a final specific activity of 150 nmol/min/mg of protein. Purification steps included several chromatography using Q-Sepharose Fast Flow, cellulose phosphate, Toyopearl HW 55 and Affi-Gel Blue. The purified PI kinase had an estimated molecular weight of 80,000 by gel filtration and 76,000 by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The purified kinase phosphorylated only PI and did not phosphorylate phosphatidylinositol 4-phosphate or diacylglycerol. Km values for PI and ATP were found to be 115 and 150 microM, respectively. The enzyme required Mg2+ (5-20 mM) or Mn2+ (1-2 mM) for activity, was stimulated by 0.1-1.0% (w/v) Triton X-100, and completely inhibited by 0.05% sodium dodecyl sulfate. The enzyme activity showed a broad pH optimum at around 7.4. The enzyme utilized ATP and not GTP as phosphate donor. Nucleoside triphosphates other than ATP and diphosphates significantly inhibited the kinase activity. However, inhibitory effects of adenosine, cAMP, and quercetin were weak.